AD_____

Award Number: W81XWH-10-1-0209

TITLE: Cyclin D1-AR crosstalk: Potential Implications for Therapeutic Response in Prostate Cancer

PRINCIPAL INVESTIGATOR: Matthew Schiewer

CONTRACTING ORGANIZATION: Thomas Jefferson University
Philadelphia, PA 19107

REPORT DATE: April 2012

TYPE OF REPORT: Annual Summary Report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
01-04-2012	Annual Summary	1 APR 2011 - 31 MAR 2012

4. TITLE AND SUBTITLE
Cyclin D1-AR crosstalk: Potential Implications for Therapeutic Response in Prostate Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-10-1-0209

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Matthew J. Schiewer

E-Mail: matthew.schiewer@kimmelcancercenter.org

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Jefferson Medical College
Philadelphia, PA 19107

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Prostate cancer is dependent on androgens and the androgen receptor (AR) for disease initiation, maintenance, and progression. Through work by our group and others, it has been shown that there is significant crosstalk between AR and the cell cycle machinery. Most importantly for our study, AR has been shown to induce the G1 to S phase transition in part via regulation of cyclin D1. Cyclin D1 serves as a rheostat to temper the pro-proliferative signaling of AR by directly binding to the receptor and inhibiting it's activity, thus inducing cell cycle arrest. As such, the AR-cyclin D1 crosstalk axis may serve to control the proliferative capacity of prostate cancer cells, and potentially alter the therapeutic efficacy of anti-cancer drugs. The data presented herein will demonstrate that cyclin D1 status does not impinge on the biological outcome *in vitro* of taxane-based therapy.

15. SUBJECT TERMS
androgen receptor, prostate cancer, cyclin D1

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	9	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Introduction

Prostate cancer (PCa) is the most frequently diagnosed and second leading cause of cancer death in men in the United States(1). The activity of the androgen receptor (AR) is critical for the development and progression of PCa, and as such is the therapeutic target of disseminated disease(2). AR drives cell cycle progression, at least in part, by regulating mTOR-dependent translation of cyclin D1, which is a critical facet of the G1-S transition machinery(3). Work from our lab and others have determined that cyclin D1 can serve as a negative feedback regulator of AR by directly binding to the receptor and inhibiting its transcriptional activity(4,5,6). The purpose of the proposed studies was to determine the effects of commonly used therapeutics (ionizing radiation, IR; docetaxel, DCTX) on the expression of cyclin D1 protein and subsequent AR regulation, as well as to determine the *in vivo* consequence of disruption of the ability of cyclin D1 to impinge upon AR signaling.

Body

Statement of work

Task 1: Determine the contribution of cyclin D1 down-regulation on response to IR and DCTX. (months 1-12).

A. Determine what residues contribute to cyclin D1 degradation in response to treatment (Months 1-6).

As demonstrated in our first annual report, threonine 286 is dispensable for cyclin D1 degradation in response to DCTX while leucine 32 is required.

B. Examine the effect of cyclin D1 status on response to therapies with regard to AR activity, cell proliferation/survival (months 6-12).

As demonstrated in our first annual report, IR and DCTX treatment of PCa cells corresponded with increased levels of prostate specific antigen (PSA) mRNA, which is a direct AR target gene that is frequently used in the diagnosis and management of PCa. Additionally, it was determined that cells deficient in cyclin D1 as achieved through RNAi technology also corresponds to increased levels of PSA transcript. New data presented in **Figure 1** demonstrate that upon treatment of either LNCaP or C4-2 cells expressing either wild type or mutant (L32A) cyclin D1 with DCTX, there is no discernable difference in cell cycle profiles, regardless of cyclin D1 allele present.

Figure 1

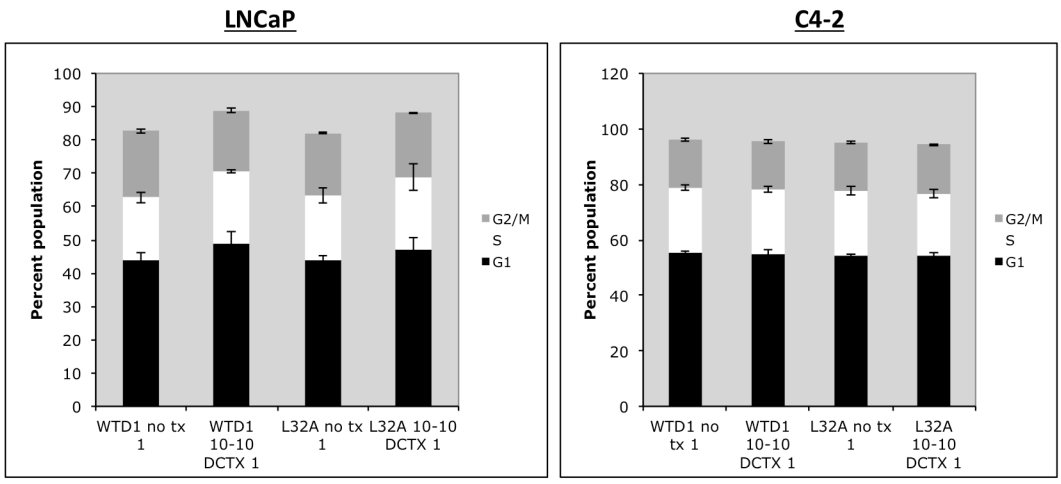

Figure legend: Indicated cell lines were transfected with indicated cyclin D1 alleles. 48 hours after transfection, cells were treated with 1x10^-10 DCTX. 24h later, cells were harvested by trypsinization, fixed, DNA stained with propidium iodide, and prepped for FACS analysis. Data represents at least three independent experiments, mean ± SD.

Correspondingly, upon knockdown of cyclin D1 in LNCaP cells and subsequent treatment with DCTX, there is no difference in cell number over time, regardless of cyclin D1 status, as shown in **Figure 2**.

Figure 2

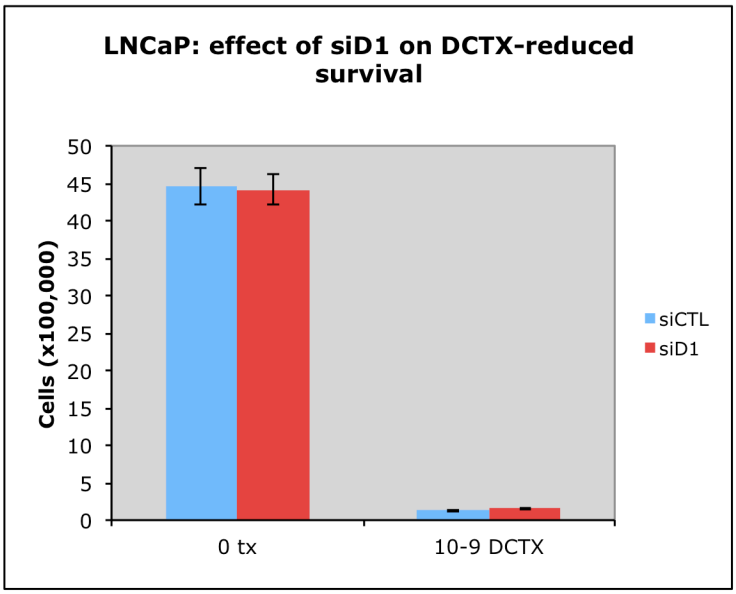

Figure legend: LNCaP cells were seeded at equal density, transfected with either control siRNA, or siRNA targeting cyclin D1. 24h later, the cells were treated with 1nM DCTX, and permitted to grow for 96h, at which time cells were harvested and counting using a hemacytometer and trypan blue exclusion. Data represents at least three independent experiments in triplicate, mean ± SD.

To examine the comparative effects of the residues of cyclin D1 found to be differentially required for DCTX-induced degration, a similar experiment was performed in both LNCaP and C4-2 cells, and relative cell cycle distribution was determined, as well as cell death as measured by sub-G1 DNA content. As shown in **Figure 3**, DCTX resulted in alterations of cell cycle in cells expressing both alleles. However, there was no statistical difference in the DCTX treated cells regardless of cyclin D1 allele expressed (T286A, which was degraded in response to DCTX, or L32A, which was refractory to DCTX-induced degradation). Together, these data demonstrate that cyclin D1 degradation induced by DCTX does not effect the biological effects of these agents in LNCaP or C4-2 cells. Currently, we investigating the effect of cyclin D1 status on the biological effect of IR.

Figure 3

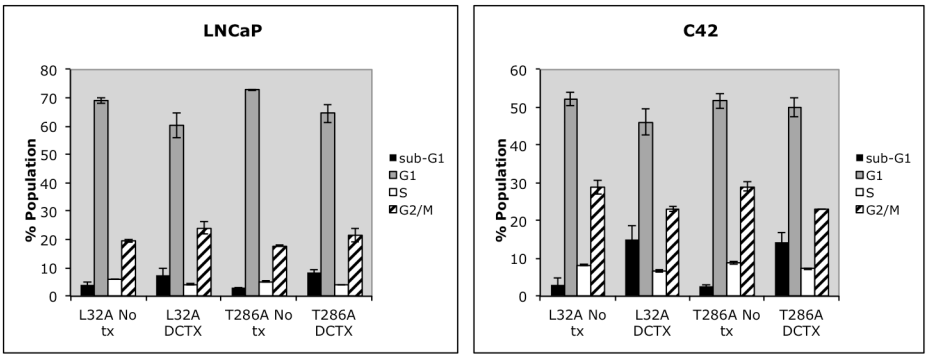

Figure legend: Indicated cell lines were transfected with indicated cyclin D1 alleles. 48 hours after transfection, cells were treated with 1x10^-10 DCTX. 24h later, cells were harvested by trypsinization, fixed, DNA stained with propidium iodide, and prepped for FACS analysis. Data represents at least three independent experiments, mean ± SD.

Task 2: **Dissect the *in vivo* consequence of PCa therapeutics on cyclin D1 and AR (months 18-36).**
A. Examine the expression of degradation-resistant and –proficient alleles of cyclin D1 in xenograft model
(months 18-21)
B. Randomize mice in 6 groups: intact/untreated group, intact/IR treated, intact/DCTX treated, castrated/untreated, castrated/IR treated and castrated/DCTX treated. Measure tumor volume with time in mice from untreated groups
(months 21-22)
C. Measure tumor volume in treated groups and monitor AR activity (serum PSA)
(months 21-22)
D. Excise tumors at the end of the experiment and determine Ki-67 indices, apoptotic indices (TUNEL) and cyclin D1 levels
(months 22-36).
As demonstrated in the new data reported under task 1 that cyclin D1 status had no effect on cancer cell biology *in vitro*, the DCTX arms of this study have not yet been initiated. An as the determination of the effect of cyclin D1 status on the biological effect of IR is currently being investigated, the IR arms of this study also have not been initiated. However, as this report is for months 12-24, we still anticipate achieving our goals prior to month 36, as was set forth in the SOW.

Task 3: Determine the cooperation of the cyclin D1 AR-repressive function (RD) with cytotoxic agents.
(months 6-18)
A. Introduce RD into cell systems of clinical relevance, treat with IR and/or DCTX
(months 6-18)
B. Examine effect on AR activity, cell proliferation/survival
(months 6-18)
Prior to initiation of these studies, we sought to compare full-length cyclin D1 and RD (cyclin D1 repressor domain) with regard to PCa cell model cytotoxicity. As demonstrated in **Figure 4**, while RD results in decreased cell number over time, indicative of cytotoxicity, full length cyclin D1 demonstrates a merely cytostatic phenotype. Based on this, we are currently determining if the cytotoxic effects of RD add to the effects of cytotoxic agents.

Figure 4

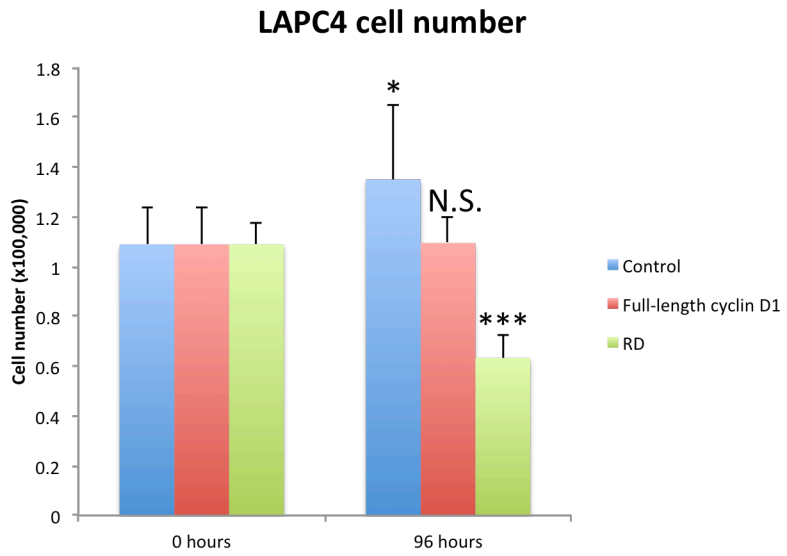

Figure legend: Cells were seeded at equal density, and infected with indicated adenoviruses. 96h after infection, cells were counted using a hemacytometer and trypan blue exclusion. Data represents at least three independent experiments ± SD. Statistical significance between 0h and 96h was determined by Student's t test: *= $p<0.05$, ***= $p<0.001$, N.S. = not significant

Task 4: Determine the in vivo consequence of cyclin D1-mediated AR regulation in the prostate
(months 1-36)
A. Generate knock-in mice (3xFLAG-tagged full-length cyclin D1 and cyclin D1-ΔRD).
(months 1-17)
As described in our previous report, these mice have been generated. Currently, we are endeavoring to breed out the ES cell selective Neomycin cassette, and increase our colony number.
B. Examine the effect of cyclin D1 on AR signaling in the prostate (months 17-36).
Ongoing and contingent upon removal of selective cassette.

Training program
Task1: Didactic coursework and laboratory research
A. Didactic coursework->completed
B. Laboratory research (months 1-36)->ongoing

Task 2: Lab meetings
Weekly (months 1-36)->ongoing

Task 3: Joint lab meetings
Bi-weekly (months 1-36)-> ongoing

Task 4: Paper of the day
Participate daily. Present bi-weekly (months 1-36) -> ongoing

Task 5: Prostate Cancer Translational Research Seminar Series
Once monthly (months 1-36) -> ongoing

Task 6: Journal club
Weekly participation. Presentation once per academic quarter (months 1-36) -> ongoing

Task 7: Conferences
A. Attend Keystone Symposia on Nuclear Receptors and present research. Completed
B. Attend annual AACR conference and present research. Completed
C. Attend other meeting of prostate cancer relevance and present research (months 12-36) -> ongoing

Task 8: Other
A. Continued interaction with researchers and clinicians (months 1-36) -> ongoing
B. Attend seminars at Kimmel Cancer Center at Thomas Jefferson University that occur multiple times per week (months 1-36) -> ongoing

Key research accomplishments:

-cyclin D1 status is not a determinant of the effects of docetaxel in LNCaP cell models

-full-length cyclin D1 elicits cytostatic effects, while RD alone elicits cytotoxic effects in PCa cell models

Reportable outcomes

Abstracts have been submitted to multiple meetings and accepted for poster presentations: AACR Advances in Prostate Cancer Research (for which I received a travel scholarship), Keystone Symposia: Nuclear Receptor Matrix: Reloaded (for which I received a travel scholarship, and the FASEB Research Conference: Integration of Genomic & Non-Genomic Steroid Receptor Actions.

Conclusion

Prostate cancer management remains a serious health concern, and understanding the mechanisms of both therapeutic efficacy and bypass are of great importance. Studies completed this far demonstrate that the *in vivo* biological effects of docetaxel are not dependent upon cyclin D1 status, despite the observation that there is an apparent increase in AR transcriptional activity. Ongoing studies will determine if this holds true for ionizing radiation, determine whether a portion of cyclin D1 (RD) can cooperate with these therapeutic modalities, and examine the role of RD *in vivo*, both as a means to suppress AR function therapeutically as well the role RD plays in murine prostate biology.

References

1. Jemal A, Siegel R, Xu J & Ward E Cancer statistics, 2010. *CA Cancer J Clin* **60** 277-300.

2. Knudsen KE, Scher HI (2009). Starving the addiction: new opportunities for durable suppression of AR signaling in prostate cancer. *Clin Cancer Res* **15:** 4792-8.

3. Xu Y, Chen SY, Ross KN & Balk SP 2006 Androgens induce prostate cancer cell proliferation through mammalian target of rapamycin activation and post-transcriptional increases in cyclin D proteins. *Cancer Res* **66** 7783-7792.

4. Burd CJ, Petre CE, Moghadam H, Wilson EM, Knudsen KE, 2005. Cyclin D1 binding to the androgen receptor (AR) NH2-terminal domain inhibits activation function 2 association and reveals dual roles for AR corepression. *Mol Endocrinol* **19:** 607-20.

5. Knudsen KE, Cavenee WK, Arden KC, 1999. D-type cyclins complex with the androgen receptor and inhibit its transcriptional transactivation ability. *Cancer Res* **59:** 2297-301.

6. Petre CE, Wetherill YB, Danielsen M, Knudsen KE, 2002. Cyclin D1: mechanism and consequence of androgen receptor co-repressor activity. *J Biol Chem* **277:** 2207-15.